J. Amer. Soc. Hort. Sci. 126(6):703–709. 2001.

Pyridine Nucleotide Charge Reduces Photosynthesis under Short-term Oxygen Deficiency

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Additional index words. chlorophyll fluorescence, NAD, NADP, oxygen deficiency, pyridine nucleotide charge, *Mangifera indica*

Abstract. Soil flooding reduces partial pressure of oxygen (pO_2) in the root zone and often results in a reduction in photosynthesis and growth. In greenhouse studies, rooted stem cuttings of the mango (*Mangifera indica* L.) rootstock selection 13/1 were exposed to anoxia by saturating the root zone with N_2 for up to 52 h. Reduced pO_2 in the root zone affected the energy status of the roots and particularly enhanced the phosphorylated and nonphosphorylated pyridine nucleotide charges—the ratio of reduced Nicotinamide-adenine-dinucleotides [NAD(P)H] to total Nicotinamide-adenine-dinucleotide content [oxidized $NAD(P)^+$ plus NAD(P)H]—that drive the redox reaction rates in cell metabolism. Also, the pyridine nucleotide charges in leaves were enhanced, while the photosynthetic rate decreased following reduction in pO_2 in the root zone. During up to 4 h of reduced pO_2, the ratio of internal CO_2 concentration in the mesophyll to ambient CO_2 concentration was unchanged. This implies a nonstomatal influence on photosynthesis. In addition, light saturation of photosystem II occurred at lower irradiance (470 $\mu mol \cdot m^{-2} \cdot s^{-1}$) resulting in reduced maximum photochemical efficiency below that of the high pO_2 controls. After 28 h of reduced pO_2, NAD(P) charges in the leaves returned to normal, diminishing its potential effect on net photosynthetic rate.

Reduced partial pressure of oxygen (pO_2) in the root zone leads to decreased root respiration due to the absence of the terminal electron receptor (Crawford, 1993). Subsequently, ATP synthesis and nicotinamid-adenosin-dinucleotide (NADH) oxidation decrease in the mitochondria (Crawford, 1993; Kwast and Hand, 1996). Within several hours of anoxic conditions less efficient anaerobic respiration pathways are initiated that yield significantly less energy in the form of ATP (Perata and Alpi, 1993). Limited ATP synthesis has frequently been studied (Crawford, 1993; Dry and Wiskich, 1982), but little information is available on the changes in the pyridine nucleotide charge (Backhausen et al., 1998). It has been hypothesized that lack of oxygen (anoxia) in the root zone causes an end-product inhibition of the flavoproteine-bound NADH oxidation located at the mitochondrial cristae (Möller and Lin, 1986; Peine et al., 1985). Kwast and Hand (1996) showed a significant increase in the concentration of reduced NADH and the pyridine nucleotide charge (NADH/[NAD^+ + NADH]) in isolated mitochondria exposed to anoxia.

Flooding-induced anoxia in the root zone usually causes a rapid reduction in the photosynthetic rate of mango (*Magnifera indica* L.) (Larson and Schaffer, 1991). The reduction in net photosynthesis (P_N) appears to be largely the result of stomatal closure due to enhanced ethylene or abscisic acid (ABA) production, resulting in reduced CO_2 uptake (Bradford and Hsiao, 1982). The rate of ATP and NADPH formation in the leaves constitute the driving force of photosynthesis (Gerst et al., 1994; Giersch et al., 1980; Laisk et al., 1991; Siebke et al., 1990). By comparison, a change in the availability of inorganic phosphate (P_i) in the chloroplasts has marginal effects on steady state photosynthesis under anoxia in the root zone (Rao and Terry, 1994). A number of studies suggest a major influence of the degree of ADP phosphorylation regulated via the carbohydrate concentration of plants (Rao et al., 1990) and concluded that the rate of NADP reduction regulates photosynthesis (Backhausen et al., 1998; Giersch et al., 1980; Hanning and Heldt, 1993; Heineke et al., 1991; Krömer, 1995; Laisk et al., 1991; Siebke et al., 1990; Wigge et al., 1993). In their model, the pyridine nucleotide charge [anabolic reduction charge (ARC) = NADPH/(NADPH + $NADP^+$) and catabolic reduction charge (CRC) = NADH/(NADH + NAD^+)] determines the electron transport rate in the two photosystems (PSI and PSII) in the chloroplasts. Subsequently, the increased NADPH concentration and the deficiency of $NADP^+$ could limit the photosynthetic rate under anoxia.

Lack of O_2 as electron receptor in the respiratory chain probably causes a decrease in the oxidation of pyridine nucleotides in the roots (Kwast and Hand, 1996; Peine et al., 1985). Studies in wheat (*Triticum aestivum* L.) found a shift from $NADP^+$ to reduced NADPH throughout the plant under conditions of reduced pO_2 in the root zone, and it was suggested that this increase in the pyridine nucleotide charge was responsible for reduced P_N under anoxia (Hoffmann et al., 1993; Peine et al., 1985).

In the present study we investigated the pyridine nucleotide charge in root and leaf tissue of mango under reduced pO_2 in the root zone and its association with CO_2 gas exchange in the leaves. The objective of the study was to demonstrate that an increase in

Received for publication 11 Sept. 2000. Accepted for publication 18 July 2001. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked as *advertisement* solely to indicate this fact.
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the pyridine nucleotide charge in leaves results in a nonstomatal reduction in P_N of mango under anoxia.

Materials and Methods

Rooted stem cuttings of mango (*Mangifera indica* L., rootstock '13/1') with well developed root systems and shoots that produced four to five flushes per year, were selected for uniformity from material grown in a greenhouse at the Department of Fruit Science, Humboldt University of Berlin, Germany. Two plants each were grown in 5.6-L plastic containers (40 cm in length, 3.5 cm in width, 40 cm in height) filled with sterilized quartz sand (0.3- to 0.8-mm grains in the top and 2.0- to 3.5-mm grains in the bottom third of containers) (Zude-Sasse et al., 1998). Containers were insulated and covered to maintain root temperature at 22 ± 1 °C during the course of the experiments. Plants were fertigated daily through a drip irrigation system using 0.3% Wuxal nutrient solution (6N–4P–8K, Aglukon, Düsseldorf, Germany) adjusted to pH 6.3 with H_2SO_4, and received supplementary light (500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 12 $h \cdot d^{-1}$ (HQI-TS 400 W/D, Osram, Munich, Germany).

Anoxia in the root zone was induced by injecting humidified N_2 from a gas cylinder (Messer, Griessheim, Germany) into the root containers through 5-mm-i.d. polyvinyl chloride tubing attached to three valves at the bottom of each pot. The sand in containers for control plants was injected with humidified ambient air at the same flow rate (1.5 $L \cdot min^{-1}$), which was controlled by a mass flow meter (Obold, Hofheim/Ts., Germany). During treatment, the shoots were well ventilated with fans to prevent environmental changes due to N_2 gassing out from the sand. Anoxia treatments lasted 2, 4, 28, and 52 h and were each performed on four replicate plants (total of 20 plants in 10 containers), and the experiment was conducted three times.

Chlorophyll fluorescence analysis. Chlorophyll fluorescence was determined using a Mini-Pam fluorometer (Walz, Effeltrich, Germany) for pulse amplitude modulated measurements (White and Critchley, 1999). A halogen lamp (Osram) was used to produce photosynthetically active [actinic (A)] radiation (*PAR*) (400 to 700 nm) of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. To achieve light saturation (S), 0.8-s pulses at an irradiance of 5000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were provided at 30-s intervals (Zude-Sasse, 1999). During measurements, chlorophyll fluorescence was continuously recorded digitally by computer. Light saturation curves were recorded before anoxia treatments according to Gerst et al. (1994). Recordings on anoxia treated and nontreated aerated control plants were taken at noon on leaves that were first adapted to dark conditions for 15 min.

Maximum photochemical yield (y) of dark-adapted leaves was determined by calculating the quotient of variable ($F_V = F_M - F_0$) to maximum fluorescence ($y = F_V/F_M$). Changes in the electron transport rate (ETR) in response to anoxia were determined on light adapted leaves by multiplying the efficient quantum yield [$F = (F_M' - F_T)/F_M'$] with the actual PAR, using a factor of 0.84 for leaf absorption and a factor of 0.5 to account for the two photosystems (ETR = $F \cdot PAR \cdot 0.84 \cdot 0.5$).

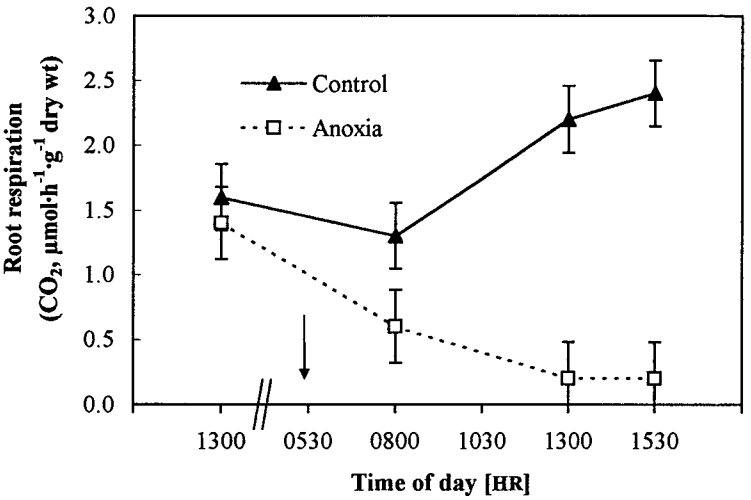
CO_2-gas exchange. Carbon dioxide and H_2O vapor exchange of attached, mature (2- to 5-month-old) leaves (n = 24) were measured with a portable photosynthesis system (CI-301PS, CID Inc., Vancouver, Wash.) and a 11-cm leaf cuvette (CI-301LC-2, CID) at 500 $\mu mol \cdot m^{-2} s^{-1}$. P_N under steady-state conditions, internal CO_2 concentration (c_i), ambient CO_2 concentration (c_a = 375 $\mu mol \cdot mol^{-1}$), and stomatal conductance were calculated automatically by the CI-301PS program functions.

Root respiration. Root respiration measurements were performed on 5-cm-long root segments (n = 6) that were excised 1 cm from the root tip and enclosed in a 50-mL cuvette, using an infrared CO_2 gas analyzer (ADC, Herts, United Kingdom) (Lambers et al., 1991). Air volume in the soil was determined at the end of each experiment as the difference between actual soil water content and saturated water content. The partial pressure of O_2 in the root zone was measured with a paramagnetic oxygen analyzer (PMA 10, M&C Products, Ratingen, Germany).

Pyridine nucleotide content. NAD and NADP levels in the roots were determined enzymatically (Brinkman et al., 1973; Zhao et al., 1987; Zude-Sasse and Lüdders, 2000). The oxidized $NADP^+$ is stable at low pH, whereas reduced NADPH is present under alkaline conditions. For NAD^+ and $NADP^+$ analyses, 5 g of the fine root system of each plant was extracted with 20 mL perchloric acid. NADH and NADPH were extracted in KOH. After extraction, samples were homogenized (Ultra-turrax T25, Jahnke&Kunkel Labortechnik, Staufen i. B., Germany), heated (60 °C) for 30 min, and centrifuged for 10 min at 3500 g_n (Labofuge GL, Kendro Laboratory Products, Hanau, Germany). Supernatants of oxidized pyridine nucleotides were neutralized with KOH and adjusted to pH 7.4 with Tris-HCl at 1 $mol \cdot L^{-1}$. Supernatants of the reduced pyridine nucleotides were neutralized with HCl and adjusted to pH 7.6 with tri-ethanolamine (TEA) at 1 $mol \cdot L^{-1}$ (Zhao et al., 1987).

Nonphosphorylated and phosphorylated pyridine nucleotides were assayed within 2 h measuring the specific enzymatic reduction of NAD and NADP with alcohol dehydrogenase and glucose-6-phosphate dehydrogenase, respectively (Brinkman et al., 1973; Zude-Sasse, 1999). A coupled colorimetric reaction with 2,5-cyclohexadien-1-one,2,6-dichloro-4-[(4-hydroryphenyl)-imino] and phenazinium,5-methyl,methyl sulfate was recorded spectrophotometrically (SP8-300, Unicam Chromatography, Cambridge, United Kingdom) at 625 nm. All chemicals and enzymes were purchased from Boehringer-Mannheim (Hoffmann-La Roche, Basel, Switzerland). The pyridine nucleotide concentration of samples was calculated based on 83% recovery and extinction of

Fig. 1. Effect of reduced pO_2 in the root zone on root respiration of '13/1' mango rootstock. Anoxia was induced at 0500 HR (↓), the light period started at 0600 HR. Vertical bars indicate SE (n = 4).



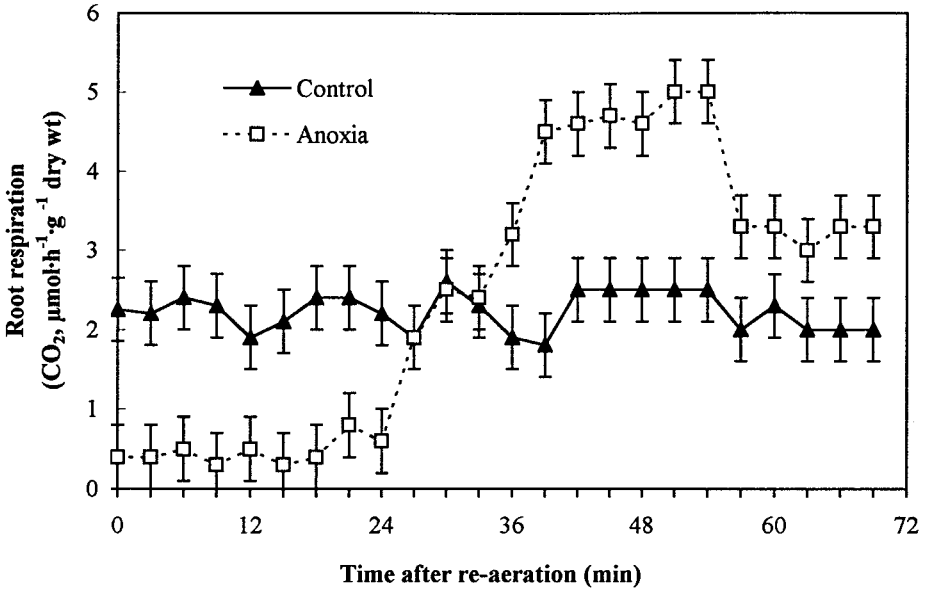


Fig. 2. Root respiration of '13/1' mango rootstock following re-aeration of the root zone after 8 h of anoxia. Vertical bars indicate SE (n = 6).

standard pyridine nucleotide solutions (Zude-Sasse, 1999). Redox potential (E_h) of the NADPH/NADP$^+$ redox system was calculated from the NADP concentration.

Experiments were analyzed as a randomized complete block design with four replications. All data were analyzed statistically using analysis of variance procedures or regression analysis (SAS Software, SAS Inst. Inc., Cary, N.C.), and means were compared using Duncan's multiple range test, $P \leq 0.05$ or 0.01.

Results

Oxygen partial pressure. The growing media had a total pore volume of 29% ± 4%. Soil water content during the experiments was 6% ± 1% and no significant water loss was measured due to flow of the saturated air or N_2 through the cuvette. The pO_2 in the control containers was 21 MPa, which was reduced by the N_2 treatment to 0 MPa within 30 s.

Root respiration. At pO_2 = 21 MPa, respiration of excised root segments increased during the day, while at reduced pO_2 root respiration decreased (Fig. 1). When the cuvettes were aerated following 8 h of anoxic conditions, root respiration rates started to increase after 23 min reaching 200% of control levels after 39 min (Fig. 2). Fifty-four minutes after the end of treatments, rates decreased again but remained higher than the control during the measuring period of 70 min.

Photosynthesis. Limited pO_2 in the root zone reduced P_N within 2.5 h (Fig. 3) and depressed the diurnal pattern of gas exchange (Fig. 4). Continued exposure to anoxia for up to 52 h further reduced CO_2 gas exchange (Fig. 3). The ratio of the CO_2 concentration in the mesophyll (c_i) and ambient air (c_a) was not affected by anoxia until 28 h when $c_i : c_a$ was increased relative to the control (Fig. 3).

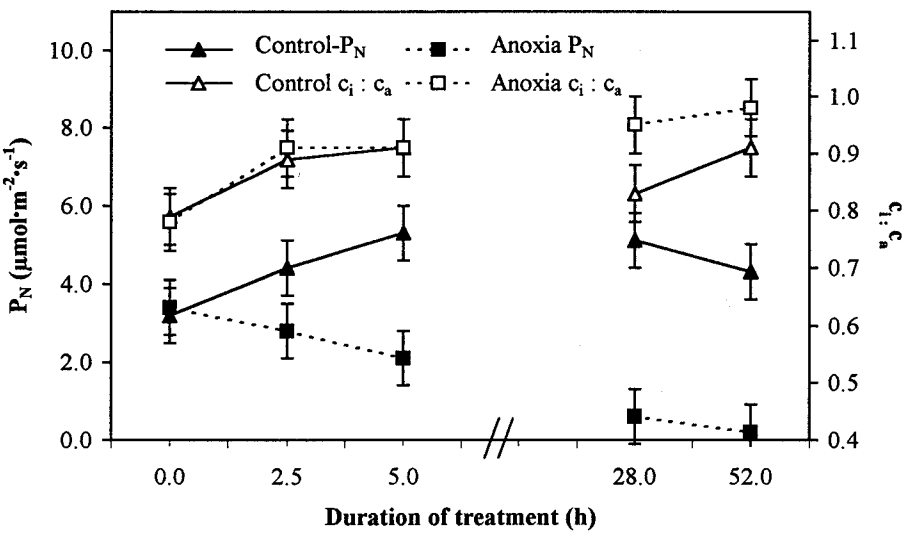
Chlorophyll fluorescence. With the duration of anoxia, dark fluorescence tended to increase from F_0 = 148 to F_0 = 208 and maximum photochemical efficiency of photosystems decreased from y = 0.81 to y = 0.70 within 2 h. Although variable and statistically nonsignificant, F_0 increased by 26% while y was reduced by 8% after 4.5 h of reduced pO_2. Maximum effective photochemical efficiency of greenhouse acclimated mango plants was derived from the inflection point of the regression curve for electron transport rate (ETR) and was reached at 550 ± 30 μmol·m^{-1}·s^{-1} (Fig. 5). At irradiances >480 μmol·m^{-2}·s^{-1}, ETR was significantly limited by reduced pO_2 in the root zone.

Pyridine nucleotide content. Under nonstressed conditions, the nucleotide content and reduction charge of NADP (ARC) and NAD (CRC) in leaves were increased by light conditions but not in roots (Table 1). Two days of anoxia in the roots caused increased concentrations of NADP and reduced concentrations of NAD in the leaves (Table 2). However, in roots both, NADP and particularly NAD concentrations were increased compared to nonstressed mango plants. Anoxia resulted in increased ARC and CRC throughout the plant (Table 3). After 4 h of anoxia the redox potential (E_h) of the NADP redox system decreased from –315 mV to –324 mV in the roots and from –312 mV to –320 mV in the leaves.

Discussion

Use of nitrogen gas instead of water to replace soil O_2 to study anoxia has several advantages: pO_2 in the soil is reduced within a very short time, and there are no changes in soil microbial respiration that could affect root respiration measurements (Trolldenier and Hecht-Buchholz, 1984; Zude-Sasse et al., 1998).

Fig. 3. Effect of short-term and midterm reduced pO_2 in the root zone of '13/1' mango rootstock on net photosynthesis (P_N) and the ratio of CO_2 in the mesophyll (c_i) and in the ambient air (c_a). Anoxia was induced at 0500 HR. Vertical bars indicate SE (n = 4).



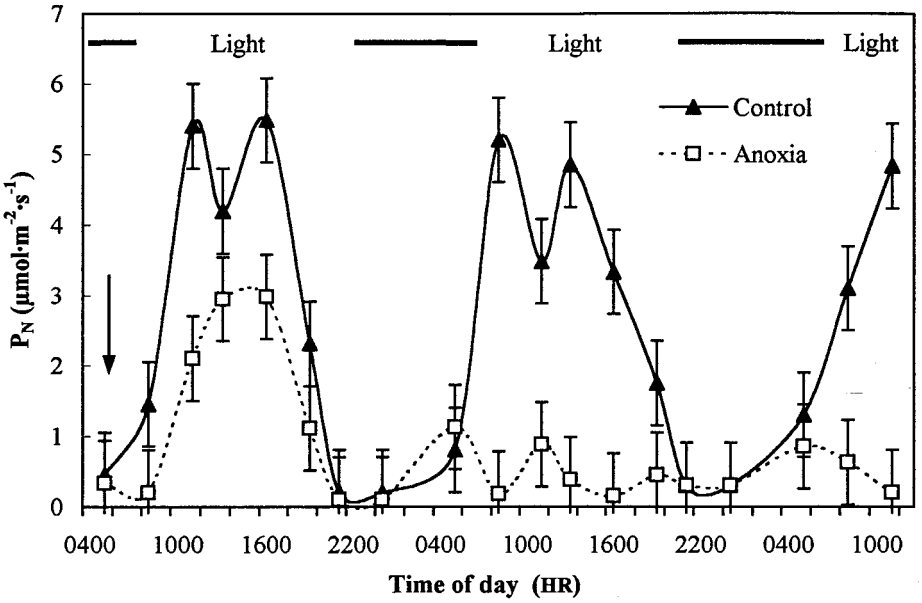


Fig. 4. Effect of reduced pO_2 (24 h) on diurnal pattern of net photosynthesis (P_N) of '13/1' mango rootstock. Anoxia was induced at 0500 HR (↓). Measurements were taken at 500 µmol·m^{-2}·s^{-1} *PAR* between 0700 and 1900 HR, and 360 ± 10 µmol·mol^{-1} ambient CO_2 concentration. Vertical bars indicate SE (n = 4).

Additionally, changes in mineral nutrient availability due to increased reduction potential in the root zone are averted (Larson, 1992; Ponnamperuma, 1972), and no CO_2 accumulation occurs in the root zone that could affect soil pH and respiration (McIntyre and McNeil, 1997; Summers and Jackson, 1996). Thus, the effect of anoxia on the energy status was isolated from potential side effects, and results should be more generally applicable to conditions of low pO_2 in the root zone.

Effect of anoxia on the energy status of roots. Under aerobic conditions, mango exhibited a typical diurnal pattern of root respiration that was probably a function of carbohydrate availability (Lambers et al., 1991). Under anaerobic conditions (anoxia), root respiration was severely limited by the lack of O_2 as the terminal electron receptor. Reaeration of the root zone resulted in an increase of the respiration rate above that of nontreated roots. Biemelt et al. (1998) described a similar overcompensation phenomenon for respiration rates previously reduced under anoxia. Limited respiration during anoxia depresses the energy status of roots (Crawford, 1993; Lambers et al., 1991). Reduced phosphorylation of ADP to ATP under anoxia has been attributed to reduced oxidative phosphorylation in mitochondria (Pradet and Raymond, 1983). This reduction in carbon metabolism (Crawford, 1993; Hand and Hardewig, 1996) was associated with reduced root growth (Lambers et al., 1991).

Under anoxia, oxidation of NADH in the mitochondria (respiration) was diminished. In isolated mitochondria, a dramatic increase in the NAD charge was found within 10 to 30 min (Kwast and Hand, 1996; Paul and Schneckenburger, 1996; Wigge et al., 1993) with the redox system of NAD almost completely reduced under anoxia.

In our experiments, an increased NAD and NADP charge in the roots was found after 2 h of reduced pO_2. During aerobic respiration as well as fermentation processes, 2 mol of NADH are oxidized per mol of glucose. After 400 min of anoxia, fermentation in wheat reached a level that could affect oxidation of NADH (Brändle, 1991; Brändle personal communication, 1999). We measured short-term and midterm (28 h) increases of CRC in the roots (Table 3). The reduction of NAD^+ to NADH during glycolysis was assumed to continue under anaerobic conditions (Drew, 1997; Perata and Alpi, 1993). We did observe a slight compensation for the NAD reduction rate through fermentation.

NADP is reduced during direct oxidation of glucose in the cytoplasm (pentose phosphate cycle), whereas NADPH is oxidized enzymatically without involvement of O_2. This oxidation has been associated with lipid synthesis in the peroxisomes (Kennedy et al., 1992; Pfister-Sieber and Brändle, 1994; Ricard et al., 1994). Activation of this oxidative pathway is likely one metabolic adaptation to anoxia (Andrews et al., 1994). The rate of NADP reduction has been recognized as an indicator for tolerance of a plant to anoxia in the root zone (Kennedy et al., 1991; Rumpho and Kennedy, 1983a, 1983b). In the present study, low pO_2 in the roots decreased overall metabolism, which was associated with reduced NADP-oxidation. A short-term increase of the NADP reduction rate could possibly be alleviated temporarily by an increased protein and lipid production for anaerobic metabolism.

The accumulation of pyridine nucleotides in their reduced form increased the reduction pressure calculated as redox potential. Pyridine nucleotides represent the terminal electron donor for root metabolism (E_0 = –320 mV) (Hanning and Heldt, 1993). Under reduced pO_2, reactions that involve electron transfer from NADH to an enzyme or metabolite are more exergonic. This was confirmed in the present study by a decreasing NADP redox potential from –315 to –324 mV under reduced pO_2. However, this reduction pressure cannot be utilized in high amounts by enzymatic pathways due to decreased carbohydrate metabolism in the citrate cycle under anoxia. Particularly, activity of reductases could be enhanced by the excess energy source (Popova and Pinheiro de Carvalho, 1998; Scheibe, 1991; Zude-Sasse and Lüdders, 2000).

Fig. 5. Effect of reduced pO_2 in the root zone for 4.5 h with increasing photosynthetic photon flux (*PPF*) on the electron transport rate (ETR) in leaves of greenhouse-grown '13/1' mango. Light saturation was derived from the inflection point of the regression curve (↑, 550 ± 30 µmol·m^{-2}·s^{-1}). Vertical bars indicate SE (n = 4).

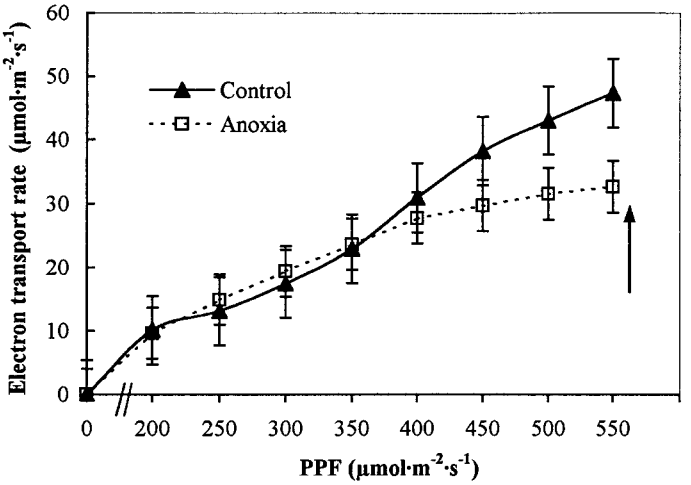


Table 1. Pyridine nucleotide content of nonstressed roots and leaves of '13/1' mango, and NADP charge (ARC) and NAD charge (CRC) measured under light (1100 HR) and dark (2100 HR) conditions (n = 4).

		Nucleotide content (nmol·g^{-1} fresh wt)					
Tissue	Treatment	NADP	NADPH	ARC	NAD	NADH	CRC
Leaves	Light	134[z]	124	0.48	390	230	0.37
	Dark	180**	114*	0.39	362**	136**	0.27
Roots	Light	143	102	0.41	207	104	0.33
	Dark	112	200	0.64	240	72	0.23

[z]Mean separation within columns for leaves or roots by Duncan's multiple range test at $P = 0.05$ (*) or 0.01 (**).

Table 2. Relative change of pyridine nucleotide content of leaves and roots of '13/1' mango under conditions of reduced pO_2 in the root zone (n = 4).

		Before treatment		After 28 h of treatment	
Tissue	Treatment	NADP (%)	NAD (%)	NADP (%)	NAD (%)
Leaves	Control	100[z]	100	100	100
	Anoxia	130*	106	182**	68**
Roots	Control	100	100	100	100
	Anoxia	113	128	198**	183**

[z]Mean separation within columns for leaves or roots by Duncan's multiple range test at $P = 0.05$ (*) or 0.01 (**).

Table 3. Effect of 2, 4, and 28 h of reduced pO_2 in the root zone on NADP charge (ARC) and NAD charge (CRC) in roots and leaves of '13/1' mango. Anoxia was started at 0500 HR (n = 4).

		Reduced pO_2 in the root zone					
		2 h		4 h		28 h	
Treatment	Tissue	ARC	CRC	ARC	CRC	ARC	CRC
Leaves	Control	0.39[z]	0.27	0.38	0.34	0.39	0.29
	Anoxia	0.44*	0.35*	0.53*	0.48*	0.43	0.35
Roots	Control	0.54	0.45	0.46	0.39	0.52	0.42
	Anoxia	0.79*	0.57*	0.57*	0.54**	0.59	0.56*

[z]Mean separation within columns for leaves or roots by Duncan's multiple range test at $P = 0.05$ (*) or 0.01 (**).

EFFECT OF ANOXIA ON THE PYRIDINE NUCLEOTIDE CHARGE IN LEAF TISSUE. The short-term increase of the NAD and NADP charge in the roots was associated with an increase of NAD and NADP charge in leaves of mango (Table 3). Increased total NADP concentration in the leaf (Table 2) was possibly a result of enhanced ARC and could be interpreted as compensation for the shortage of $NADP^+$. This supports our hypothesis that $NADP^+$ is deficient under anoxia. Similarly, NADP concentrations typically increase with the onset of photosynthesis in light (Gerst et al., 1994; Kitzmann, 1996; Rao et al., 1990; Wigge et al., 1993). Another potential mode of action could involve transhydrogenase that shifts total pyridine nucleotides from NAD to NADP (Peine et al., 1985; Rao et al., 1990).

EFFECT OF ANOXIA ON CO_2 EXCHANGE OF THE LEAVES. Carbon dioxide exchange of mango leaves was reduced significantly under anoxia (Fig. 3). Although this phenomenon has been shown in a number of studies (Andersen et al., 1984; Beckman et al., 1992; Bradford and Hsiao, 1982; Larson, 1992; Larson and Schaffer, 1991; Pezeshki et al., 1996; Vu and Yelenosky, 1991), the reasons for this are still unclear (Schaffer et al., 1994).

STOMATAL EFFECTS ON PHOTOSYNTHESIS. Reduced stomatal conductivity is commonly discussed as the cause for reduced CO_2 exchange under anoxia (Jackson and Drew, 1984; Pezeshki et al., 1996; Schaffer et al., 1994). Under anoxia, however, c_i is either increased (Vu and Yelenoski, 1991) or unchanged (Beckman et al., 1992; Pezeshki et al., 1996). In mango, we measured a temporary reduction in stomatal conductance that was associated with a reduction in photosynthesis. However, we did not find that limited P_N was triggered by a reduction in c_i (Fig. 3). Although our method cannot rule out heterogeneity of stomatal opening (patchy stomatal conductance), mango leaves appear homobaric (Terashima et al., 1988). Thus, reduced stomatal conductance under low pO_2 in the root zone is a consequence, rather than the cause of a reduced CO_2 exchange in mango.

In studies lasting days to weeks, decreasing hydraulic conductivity of roots and subsequent changes in the water status may be more relevant for the response to flooding (Freundl et al., 1998; Syvertsen et al., 1983). A limited water flow from the roots would eventually force stomatal closure and constrain CO_2 exchange of the leaves. In short-term flooding studies, however, nonstomatal factors such as the pyridine nucleotide charge may limit photosynthetic rate.

NONSTOMATAL EFFECTS ON PHOTOSYNTHESIS. After an initial increase, reduction rates of phosphorylated and nonphosphorylated pyridine nucleotides in the leaves decreased to near control level within 28 h of anoxia. Endogenous mechanisms may regulate the electron gradient to maintain an effective cell metabolism. In the photochemical reaction chain, plastoquinone (Q_A) is the electron receptor following PS II (Haumann and Junge, 1999). ETR is limited when Q_A and the electron transport chain via ferredoxin ($E_0 = -444$ mV) to NADP ($E_0 = -320$ mV) is already in its reduced state (Krause and Weis, 1991; Schreiber et al., 1995). Fluorescence measurements with and without addition of $NADH^+$ demonstrated directly the associated effects of NADP charge on ETR (Mi et al., 1994; Schreiber et al., 1995).

Results presented here demonstrate that, in the short-term,

nonstomatal factors limit photosynthesis under anoxia in the root zone. This was indicated by reduced effective photochemical efficiency, while dark fluorescence (F_0) was enhanced. Increased F_0 of dark-adapted leaves is generally considered to be the result of suppressed electron transport, i.e., a partially blocked electron pathway. The limited ETR with increasing irradiance implies reduced capacity of the electron transport chain. We conclude that an increased pyridine nucleotide charge is limiting the photosynthetic rate, possibly by disrupting electron transfer from ferredoxin to $NADP^+$. Over longer periods of anoxia, stomatal effects may outweigh the effects of NADP charge.

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